assay using full-length and truncation mutants of the SNARE proteins: syntaxin-1A, SNAP-25B and synaptobrevin, which have 0-4 cysteines. With this assay we are able to quantitatively measure the number of cysteine residues modified in reactions such as palmitoylation and oxidation. This assay is as simple as running an ELISA or western and should allow greater elucidation of the chemistry of cysteine residues in proteins cysteine due to its high resolution.

250-Pos Board B129

A Microfluidic Platform for the Culture & Analysis of Single Cells

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In modern biology, it is often assumed that populations of cells are composed primarily of average cells; cells that do not deviate significantly from an observational mean. This assumption is empirically convenient and until recently was necessary due to technological limitations. However, it is possible that ignorance of cellular individuality may lead one to draw incorrect conclusions, especially when the population under study is heterogeneous. Cells that exhibit significant deviation from the mean behavior can reveal important information which would be normally obscured by ensemble averaging techniques.

We have developed an array of microfluidic analytical techniques capable of studying the biochemistry of single cells [1,2,3]. Our current effort focuses on the development of a microfluidic device capable of sustaining a cell culture of a unicellular microorganism, Synechococcus, which can be resolved at the single-cell level. In our microchip, cells are captured hydrodynamically via a pressure-driven cross-flow of nutrient media. With efficient manipulation of the cellular microenvironment, the individuality of the cells' adaptive responses to stress conditions such as nutrient deprivation can be studied quantitatively using fluorescence microscopy. The design of imaging system with controlled illumination source as well as the use of different pumping mechanisms is described.

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2. Wu, H., Wheeler, A. & Zare, R.N. Proc Natl Acad Sci U S A 101, 12809-12813 (2004).
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251-Pos Board B130

A Pulse Width Modulated Microfluidic Diluter

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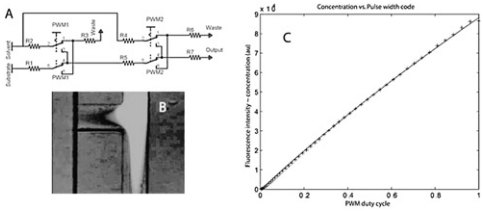
The preparation of solutions of different concentrations is often an essential part of chemical or biological assays. We have developed a pulsed microfluidic dilution concept, suitably for flexible programming of an accurate output solution concentration. Only minute amounts of chemicals are needed and dilution series with high resolution can be generated. The concept of our dilution chip is similar to digital analog conversion in electronics, using pulse width modulation (PWM).

By means of PDMS replica molding, a multi-stages PWM diluter has been constructed. Fluorescence imaging protocols with microscope/SLR camera as well as electrochemical probing with microelectrodes were employed for characterization and calibration, showing that the design allows for accurate dilution over 2 orders of magnitudes with high controllability, and at the same time minimal external components. This device concept can be applied in stand-alone diluter circuits or as a component in more sophisticated fully integrated analytical devices.



252-Pos Board B131

Controlled Near Infrared Laser-Activated Liposome Release

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Liposomes have been evaluated as drug delivery vehicles for decades. However, it is hard to prepare liposomes to both balance enhanced drug retention and rapid and targeted content release. The challenge is to initiate the release of encapsulated drugs at the diseased site at a controlled rate. We recently developed a novel photo-activated approach by which near-complete contents release from liposomes can be completed within seconds by irradiating encapsulated or tethered hollow gold nanoshells (HGN) with a near infrared (NIR) pulsed laser. The rapid heating of the gold nanoshells leads to unstable microbubble formation and collapse, the same type of cavitation events associated with ultrasound. Our approach is conceptually analogous to the use of optically triggered nano-''sonicators'' deep inside the body for drug delivery. We demonstrate that even though the local temperature surrounding HGNs can be very high, the bulk temperature of the solution only rises by ~1°C. Results from electrophoresis and quantitative PCR all show no damage to DNA molecules mixed with HGNs after NIR irradiation. These results confirm the potential of using this optical approach to permeabilize lipid membranes and facilitate the cellular uptake of DNAs for gene therapy. Since DNAs are relatively robust macromolecules, we also investigated the more delicate dye molecule, carboxyfluorescein (CF), which contains a double bond; liquid chromatography followed by mass spectral analysis shows that ~95% of CF molecules are intact. These results agree well with our hypothesis that only an few nanometer thick layer surrounding HGNs reach temperatures above the explosive boiling temperature (~ 650 K), so that only CF molecules close to HGNs are damaged. Other NIR responsive materials, such as carbon nanotubes and solid gold nanoparticles were used as triggering agent for liposome release, and their release efficiencies are compared with those of HGNs.

253-Pos Board B132

The Collaboratory for Structural Nanobiology

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Manufactured nanobiomaterials exploit the unusual properties of nanomaterials to develop new forms of intervention in biological systems. Nanobiotechnology is the field of science focused on the design, synthesis, characterization and application of nanomaterials and nanodevices to biological and biomedical problems. The success of nanobiotechnology hinges on our ability to characterize, predict, and control the biological properties of nanobiomaterials. Nanoinformatics is a collection of multi-disciplinary approaches to catalog, correlate, and model nanomaterial properties. CaNanoLab (http://cananolab.abcc.ncifcrf.gov) is an early example of a nanobioinformatics portal dedicated to foster the rapid dissemination of nanobiological information across the scientific community. Nanobioinformatics studies are complex because they must simultaneously deal with the large dispersion of chemical formulations of nanobiomaterials (ranging from polymer to metal oxide particles), the lack of a common language across contributing disciplines, and the lack of a low level language that can be used across nanoparticles. We could argue that, in lieu of a sequence space, similar to that available to bioinformatics studies of peptides and nucleotide sequences, we could build a structure based annotation and analysis of nanobioparticles that could help us cross-analyze their properties. Computer characterization of nanobioparticles is key to build a structure-based nanoinformatics infrastructure. We are in the process of building a nanobioinformatics service dedicated to the collection, curation, and correlation of structural, physico-chemical, and biological, and biomedical data: the Collaboratory for Structural Nanobiology (CSN http://csn.ncifcrf.gov). We have used CSN to explore nanobioparticles data storage, retrieval, and analysis in the context of nanobiological studies. This work has been funded in part with funds from the NCI-NIH (Contract No. NO1-CO-12400 and HHSN261200800001E). The contents of this publication do not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

254-Pos Board B133

Tunable Delivery Of Chemical Gradients Over Large Cell Culture Substrates Using Microfluidic Stacked Flows

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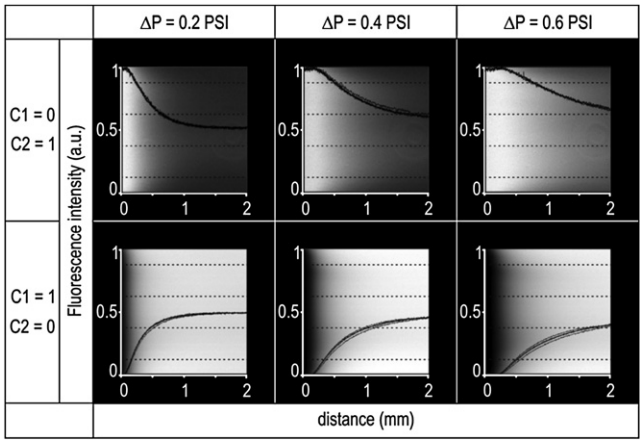
Biomolecular gradients play an essential role in studying various biological phenomena such as development, cancer, inflammation, and wound healing. This paper reports a novel microfluidic device for generating tunable biomolecular gradients over large areas on cell culture surfaces. Laminar streams are stacked above the surface to generate a steady-state gradient via diffusion in the direction orthogonal to the flow and to the surface. Finite-element modelling was used to predict negligible shear forces at the range of gradients possible by tuning flow rates. The surface gradients were characterized with fluorescence microscopy; image analysis verified the presence of a one-dimensional gradient across a 2x2 mm area. Fig. 1 shows a variety of surface gradients obtained simply by changing the inlet configuration and pressure settings. Superimposed onto these images are the linescans taken across 4 different regions of the device which demonstrate lateral uniformity (excluding edge

effects near walls <25% of area). Due to the binary branching of the inlets, the perfused area can be arbitrarily wide (here, 2 mm). This enables analyzing responses of large cell populations in parallel and acquiring rich statistics on single-cell variability.



255-Pos Board B134

Detection and Identification of Virus Particles on a Microfluidic Platform

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We have developed a nanoparticle-based assay to capture and detect targeted virus particles on a microfluidic platform. Antibody-decorated magnetic nanoparticles were used to capture virions and detection was afforded by measuring the change in magnetic birefringence relaxation rates. Vesicular Stomatitis Virus (VSV), a bullet-shaped, negative-strand RNA virus, is used as a model for air, water or blood-borne pathogenic viruses. Antibodies against the single envelope glycoprotein of either the Indiana or New Jersey strains of VSV were conjugated to 30nm superparamagnetic nanoparticles to generate the capturing reagent. The size distribution of the antibody-nanoparticle conjugates was determined using a CONTIN analysis of dynamic light scattering (DLS) data. Our results were confirmed by TEM analysis. Antibody-decorated nanoparticles were combined with one of the VSV strains and subjected to Ismagilov mixing, utilizing two immiscible phases in micro-channels to increase the speed of capture. Virus binding was determined by changes in hydrodynamic volume of the virus-nanoparticle complexes as measured by their birefringence relaxation rate under stopped flow conditions. In addition, the dynamics of particle complex formation and aggregation was studied by DLS at different scattering angles. We compared the results of these two methods for measuring time-dependent increases in the size of the nanoparticle/virus complexes. The birefringence relaxation method is more adaptable to field and other applications than DLS since it is relatively insensitive to particle concentration or to the presence of dust and sample impurities. Our approach is superior to existing techniques such as qPCR or ELISA assays due to the speed of detection and insensitivity to environmental contaminants. We are looking into the suitability of this assay for point-of-care applications.

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256-Pos Board B135

Phenotypic and Genotypic Heterogeneity of Cyanobacterial Populations in Hot Spring Microbial Mats Revealed by Microfluidic Single-Cell Analysis

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The microbial mats found at Octopus Spring in Yellowstone National Park exhibit highly organized community structures. Thermophilic unicellular cyanobacteria (Synechococcus spp.), found in the 1-mm thick top layer of these hot spring mats, act as the primary producers in the microbial communities. The cyanobacterial population is an excellent model system for studying how environmental factors affect the structure of a microbial community because the system is relatively simple and formed under a well-defined set of environmental gradients such as temperature, oxygen and light levels. To obtain a detailed description of both phenotypic and genotypic structures of the population, we extended our microfluidic approach that has been previously developed for single-cell protein analysis of a similar type of cells [Huang et al., Science, 315, 81-84 (2007)]. First, a protein analysis chip was developed for simultaneous analysis of multiple single-cell lysates for higher throughput. We demonstrate that 16 cells can be analyzed individually during each round of analytical procedures to obtain phycobiliprotein distributions at the single-cell level. Second, a genetic analysis chip was designed to amplify genomic DNA from individual cyanobacteria cells via multiple displacement amplification. The presence of a selected set of genes was compared among populations under different environmental conditions. This type of single-cell genomic data is useful for elucidating the role of cyanobacterial species deduced from the metagenomic analysis of the microbial mat samples.

257-Pos Board B136

Adsorption and Stability of Streptavidin on Cluster-Assembled Nanostructured TiO*x* Films

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The study of the adsorption of proteins on nanostructured surfaces is of fundamental importance to understand and control cell-surface interactions and, notably, cell adhesion and proliferation; it can also play a strategic role in the design and fabrication of nanostructured devices for postgenomic and proteomic applications. We have recently demonstrated that cluster-assembled nanostructured TiO*x* films produced by supersonic cluster beam deposition [1] possess excellent biocompatibility and that these films can be functionalized with streptavidin, allowing the immobilization of biotinylated retroviral particles and the realization of living-cell microarrays for phenotype screening [2,3].
Here we present a multitechnique investigation of the adsorption mechanisms of streptavidin on cluster-assembled TiO*x* films. We show that this nanostructured surface provides an optimal balance between adsorption efficacy and protein functionality. By using low-resolution protein arrays, we demonstrate that a layer of adsorbed streptavidin can be stably maintained on a cluster-assembled TiO*x* surface under cell culture conditions and that streptavidin retains its biological activity in the adsorbed layer. The adsorption mechanisms are investigated by atomic force microscopy in force spectroscopy mode and by valence-band photoemission spectroscopy, highlighting the potential role of the interaction of the exposed carboxyl groups on streptavidin with the titanium atoms of the nanostructured surface.

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[3] Carbone R. et al., Biomaterials 2007;28(13):2244(53).

258-Pos Board B137

A "Microfluidic Nose": Detection of Olfactory Sensory Neuron Responses to Odorants Across the Whole Olfactory Receptor Space

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In vertebrates, odorant molecules are detected by olfactory sensory neurons (OSNs) present in the nasal epithelium. A large, G-protein coupled receptor gene family is responsible for transducing the detection of a cognate molecule for a given receptor. Each OSN appears to express only one of thousands of olfactory receptor (OR) genes in rodents. Odorants are perceived by combinatorial activation of a number of ORs (it is specific to a subset of odorants); each OR recognizes a range of odorants and odorants are typically recognized by a number of ORs. Given the approximately thousand OSN/OR types and the hundreds of thousands of potential ligands, measuring individual OSN activation with the usual *in-vivo* and *in-vitro* methods is a laborious task that is not suitable for interrogating the whole OR space. Hence a microfluidic and high-throughput system was developed to analyze these cells.
Utilizing the techniques of soft-lithography, we developed a microwell array of ~32,000 wells (20 um diameter, 10 um depth) to capture dissociated olfactory epithelia (OE) cells and sequentially exposing them to different odorants. Cell response was detected using the Fluo4AM calcium binding dye. By imaging the fluorescence change in each well, a response profile to each odorant can be constructed for thousands of individual OSNs simultaneously.

259-Pos Board B138

SOI Nanofet Devices For Ultra-Sensitive Detection of Biomolecules

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In this work the fabrication and fluidic testing of silicon-on-insulator (SOI) field effect devices for the label-free detection of biological molecules are presented.